Analysing Chem/Bio-Markers in Saliva using a Portable Optical Detection Platform

Simon Coleman

Andrew Kavanagh, Dylan Orpen, Jung Ho Kim, King Tong Lau and Dermot Diamond

CLARITY: Centre for Sensor Web Technologies, National Centre for Sensor Research, Dublin City University, Dublin 9, Ireland

Overview

- ***Treatment and monitoring of Bipolar Disorder***
  - *Lithium therapy for mood stabilisation*
  - *Non-invasive sampling of patient*
- ***Chemo/Bio-markers for Bipolar patient monitoring***
  - *Fluorescent detection of Lithium*
  - *Colourimetric determination of α-amylase*
- ***Portable analysis of Bipolar disorder using multi-analyte platform***
- ***Detection of Chemo/Bio-markers using mobile platform***
- ***Conclusions and future applications***

Treatment and monitoring of Bipolar Disorder

Lithium treatment for mood stabilisation

- *Current treatment of Bipolar disorder involves administration of Lithium in the form of lithium carbonate tablets.*

- *Typical serum (blood) levels range between 0.6 – 1.3mmol[1] with toxic levels above 1.5mmol[1]*

➔ ***narrow therapeutic/toxic range***

<u>Two issues from current methods of monitoring dosage:</u>

1. *Blood typically drawn **<u>ONCE</u>** weekly to determine lithium levels in patient.*
   - *Risk of **<u>overdose/intoxication</u>** event being missed by infrequent monitoring*

2. *Invasive nature of blood drawing procedure.*
   - *Discomfort associated with blood sampling*

[1] R. Regenthal *et al*, J. Clin. Monit., 1999, 15, 529-544

Bipolar Disorder

Non-invasive sampling of patient

- *Invasive nature of drawing blood limits number of samples and decreases patient compliance.*
- *Interest in alternatives to Blood analysis sought for conditions requiring frequent analysis such as Diabetes.*
- *Saliva chosen due to ease of accessibility, low sample preparation requirements and minimal invasiveness.*

Blood (Top): http://www.ivteam.com/tourniquet-application-time/ <accessed 11/06/12>

Bio/Chemo-markers for Bipolar Patient monitoring

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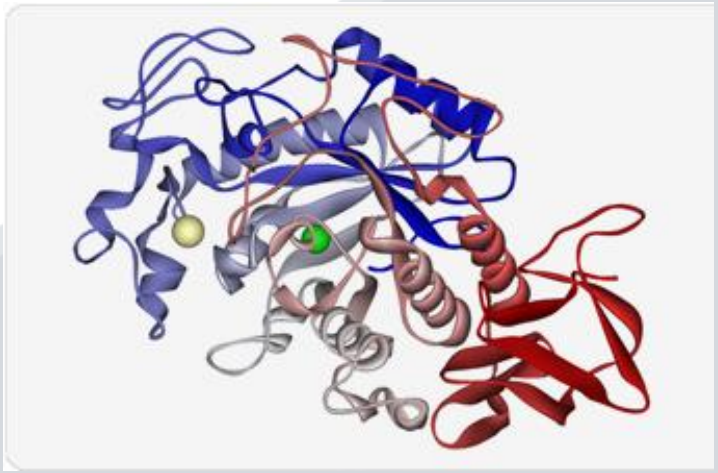
Chemical marker: Lithium

- Uncommon element in body resulting in minimal interference due to alternate sources of lithium.
- Readily found in bodily fluids during treatment; urine, saliva, blood and sweat. *Typically found to be in higher concentration in saliva than blood*

Biomarker: α-amylase

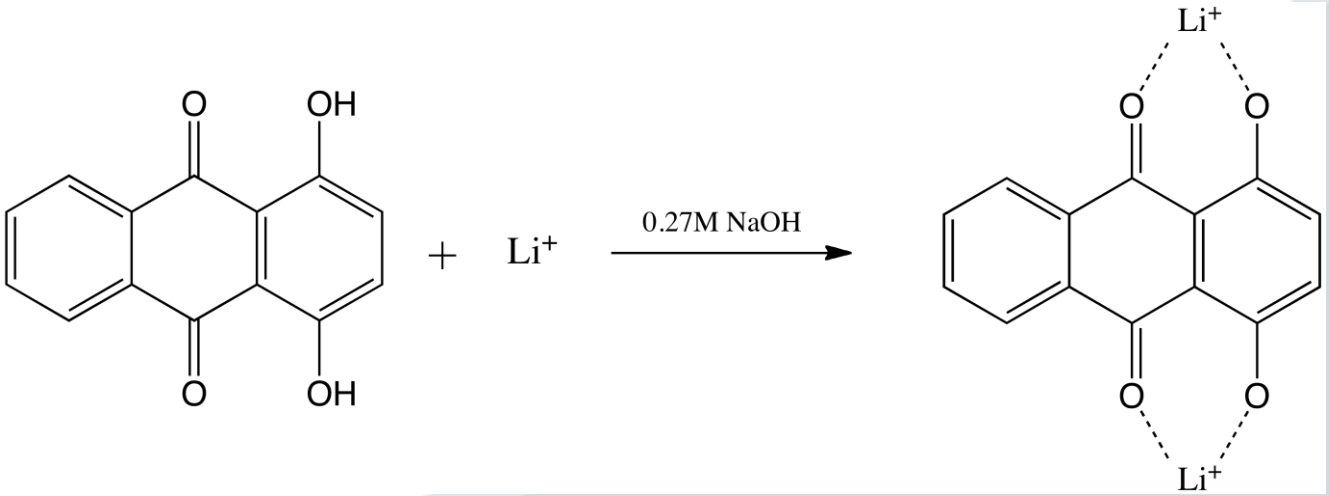
- Present in blood (Pancreatic) and saliva (salivary)
- Salivary α-amylase found to exhibit sensitivity to stress.
- Proposed use as stress monitor in conjunction with other methods of mood analysis (psychiatric assessment, vocal pattern analysis) .



Lithium (top): http://www.sciencephoto.com/image/111204/530wm/C0046993-Lithium_Carbonate-SPL.jpg <accessed 14/06/12>
Amylase (bottom): http://www.salimetrics.com/my-spit-research/analytes/a-amylase.php <accessed 11/06/12>

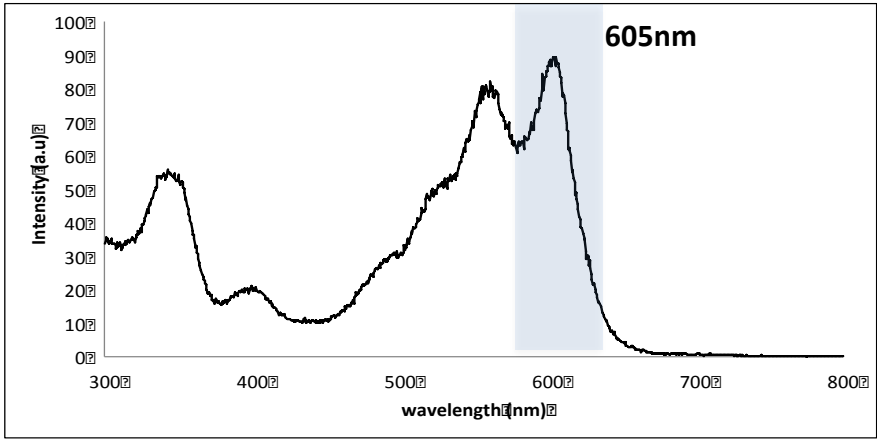
Fluorescent Detection of Lithium

- Highly sensitive method using ‘dark background’ and lithium specific probe dye.
- 1,4-Dihydroxyanthraquinone (Quinizarin) found to preferentially complex with Lithium
- Complex absorption at 605nm and corresponding emission at 624nm

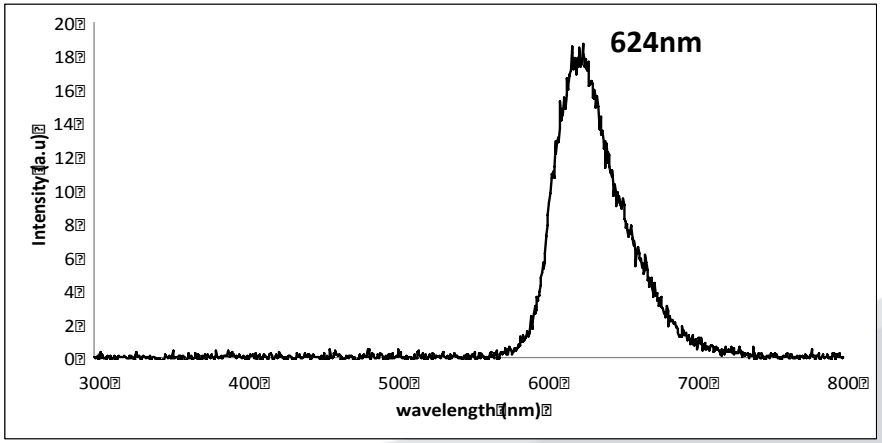


Quinizarin dye and Lithium complex formation

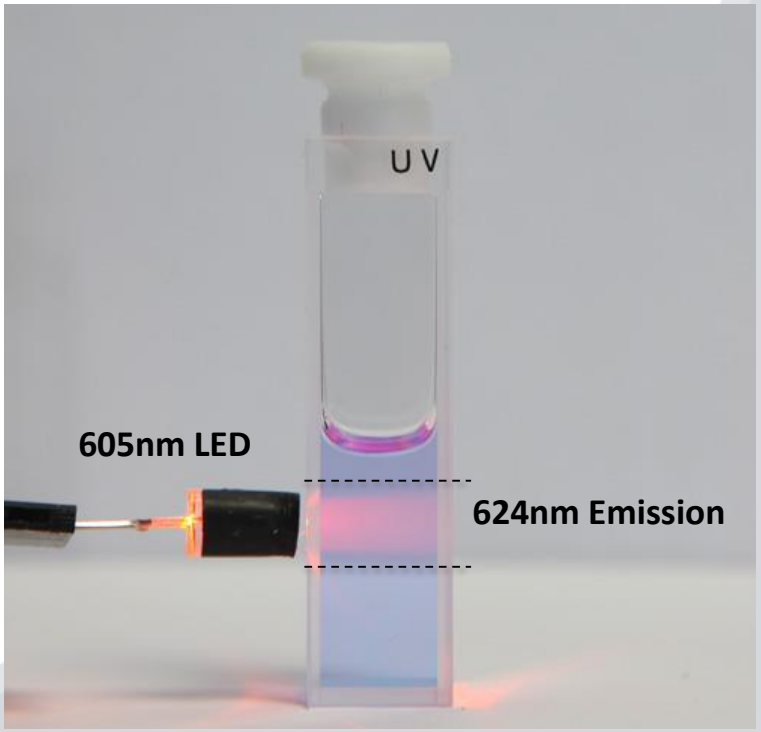
Fluorescent Detection of Lithium



Excitation spectrum of 1mM Quinizarin-lithium complex

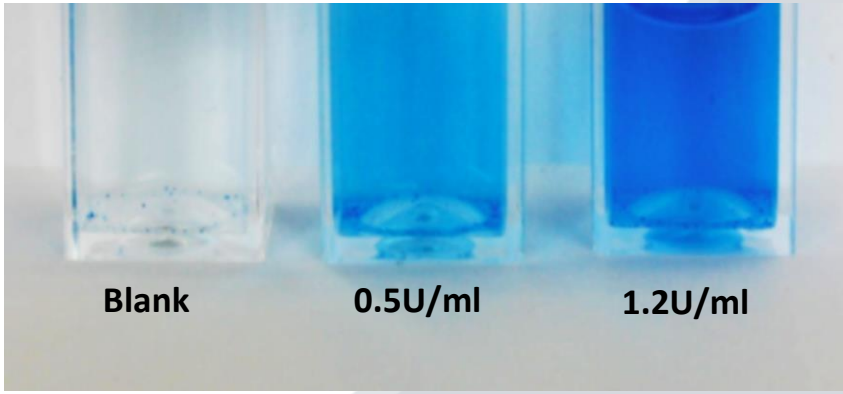


Emission spectrum of 1mM Quinizarin-lithium complex



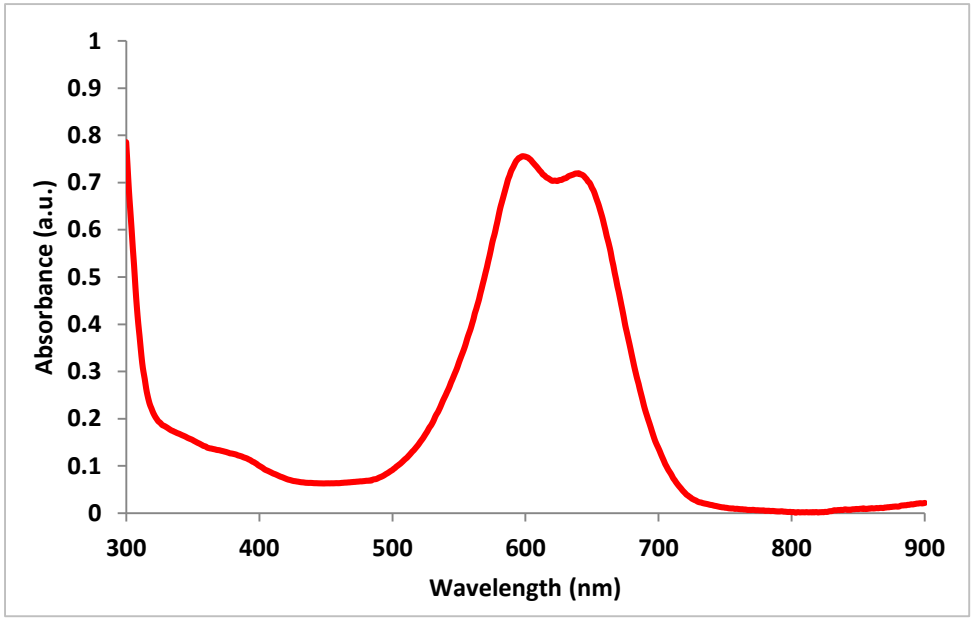
Colorimetric determination of α-amylase

- Exploitation of α-amylase enzymatic break down of starch.
- Water insoluble starch polymer with attached blue dye is added to aqueous saliva sample.
- As the starch is broken down, the dye is released into the water resulting in colour change.
- Measurement of absorption at 620nm allows for the determination of amylase concentration in saliva.

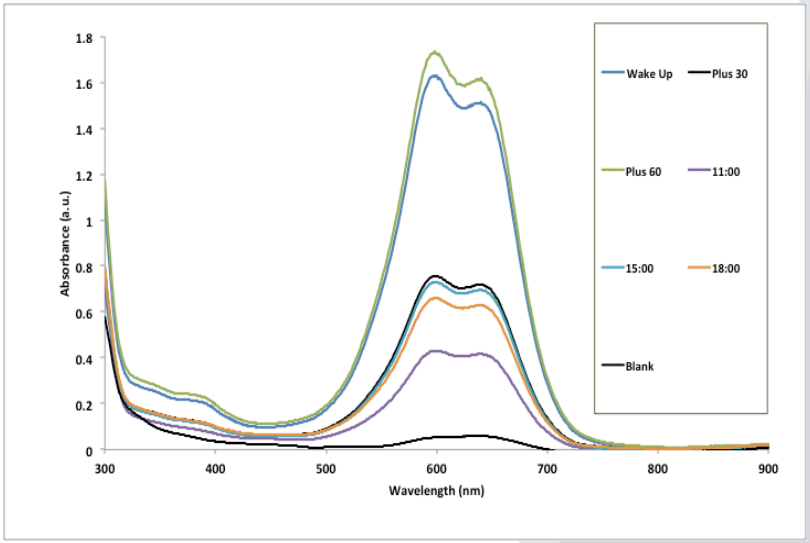


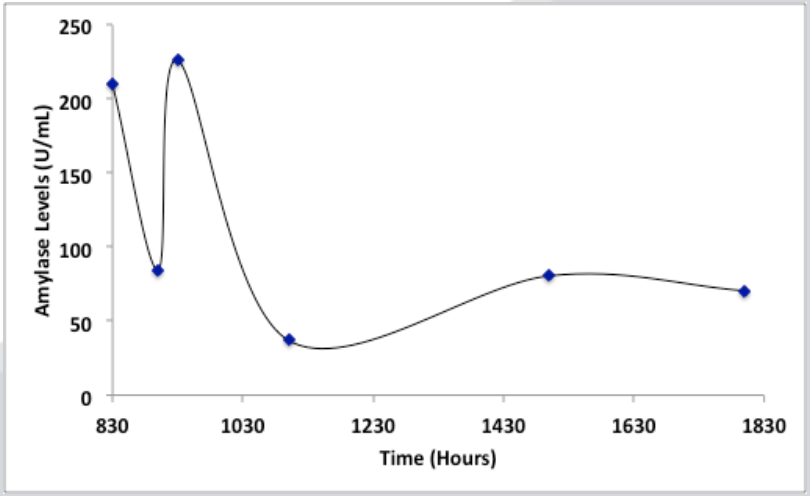
Colour change in α-amylase concentrations in response to stress

Colorimetric determination of α-amylase



Characteristic absorption spectra of released blue due following breakdown of starch polymer backbone.





Absorption changes related to varying levels of amylase present in saliva throughout a day (healthy individual)

Portable analysis of Bipolar disorder using multi-analyte platform

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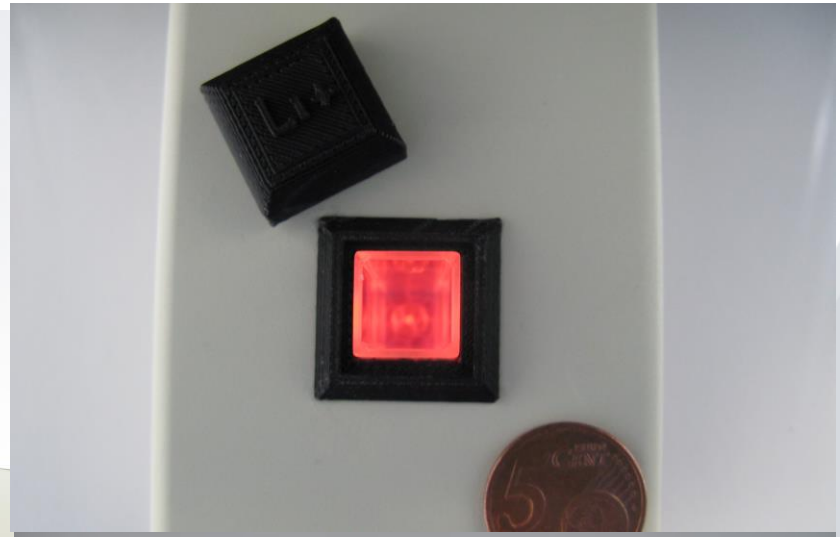
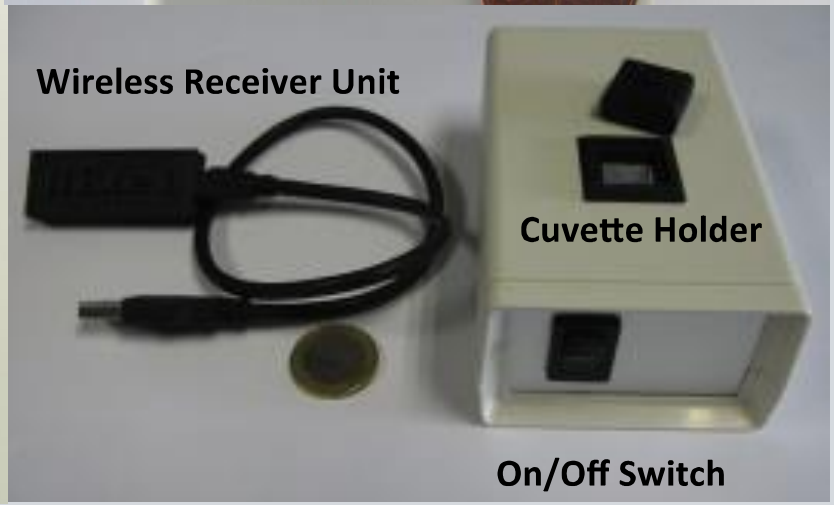
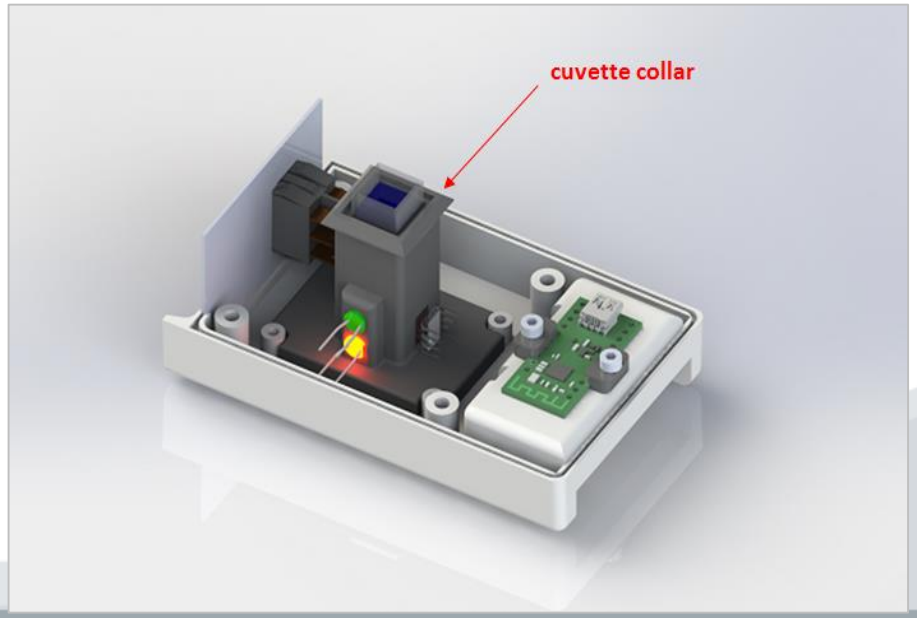


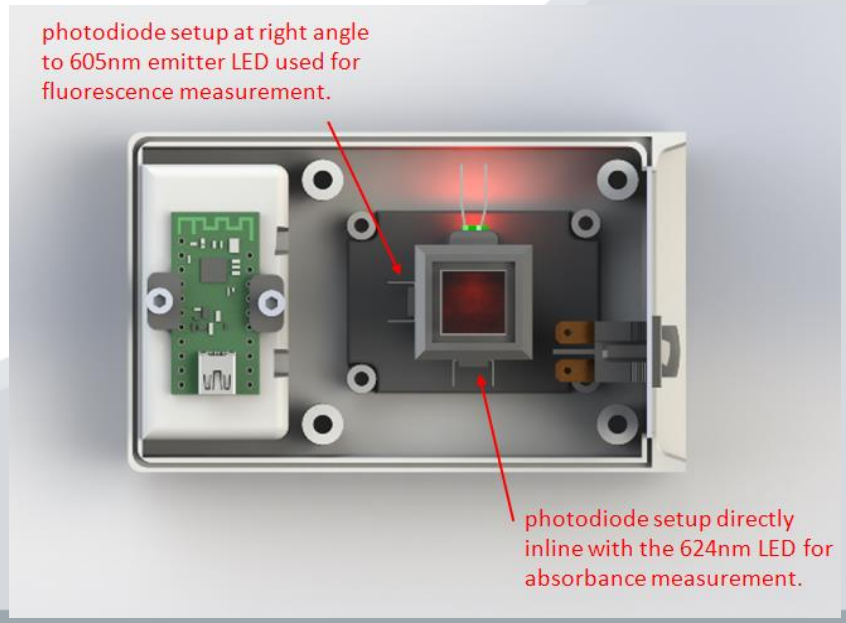


Portable analysis of Bipolar disorder using multi-analyte platform

- ***Wireless microcontroller (Wixel©) with dual LED system and individual photodiode detectors optimized for each target marker.***

- ***Automated Software programme:***
  - ***'li' (lithium) and 'aa' (α-amylase) commands initiate custom measurement routine.***
  - ***180s settling time hard coded to device for lithium analysis.***
  - ***20 individual measurements printed to screen***
  - ***Device calculates average and prints to screen.***





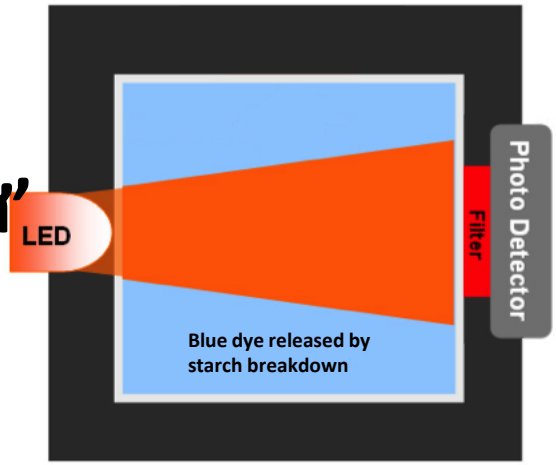
Portable analysis of Bipolar disorder using multi-analyte platform

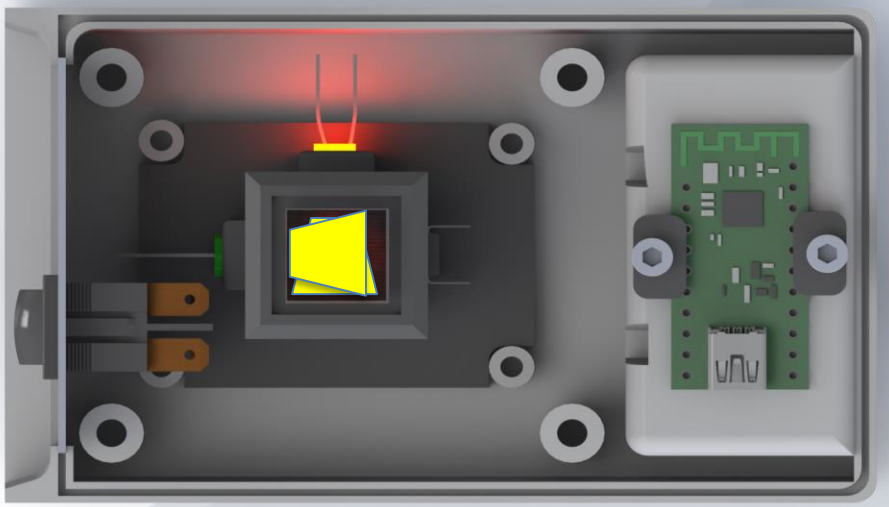
Fluorescence:

Absorbance:

Ex: 605nm

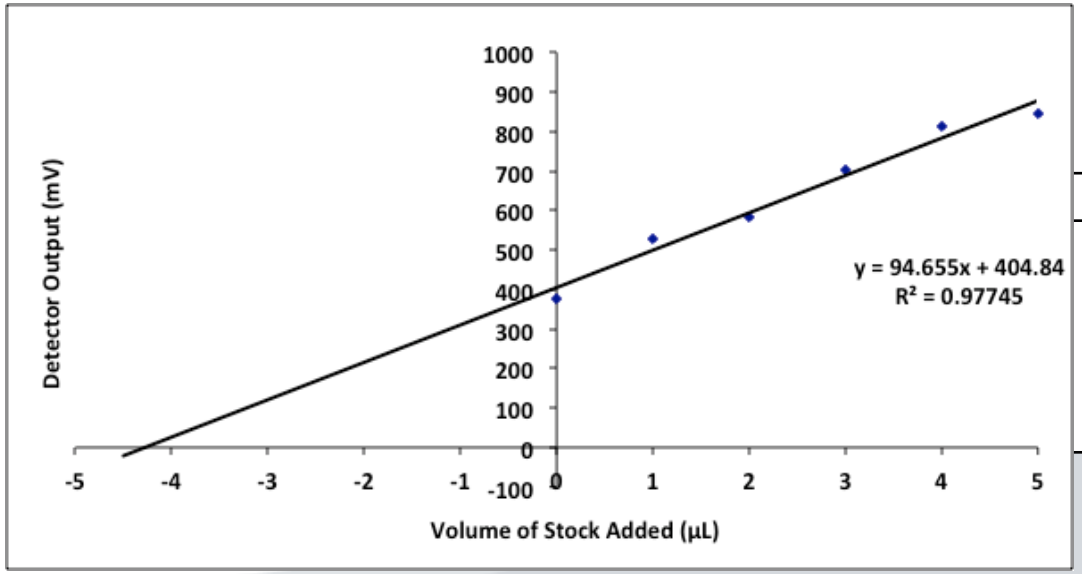
Em: 620nm





Determination of Chemo/Bio-markers using mobile platform

- Standard addition technique to determination of lithium levels:
  - Increasing addition of stock solution containing lithium of known concentration added to saliva sample containing unknown lithium concentration.
  - Validation versus standard method (ICP-AES)

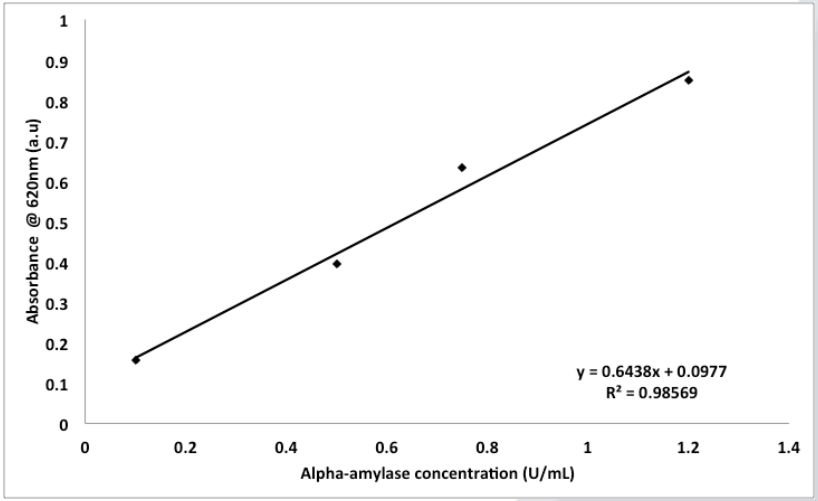


Standard addition curve for the determination of unknown saliva lithium concentration

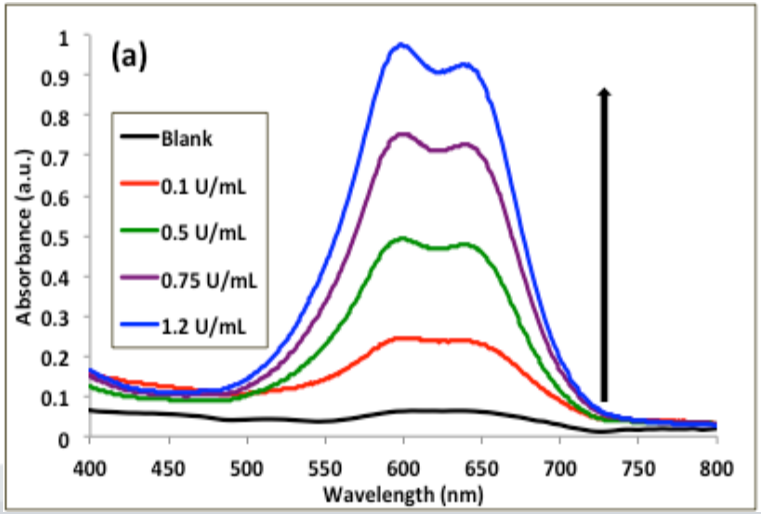
sample	*concentration (mM)*
Known saliva concentration	**1.00**
Portable platform measurement	1.06
ICP-AES measurement	0.93
standard deviation	0.09

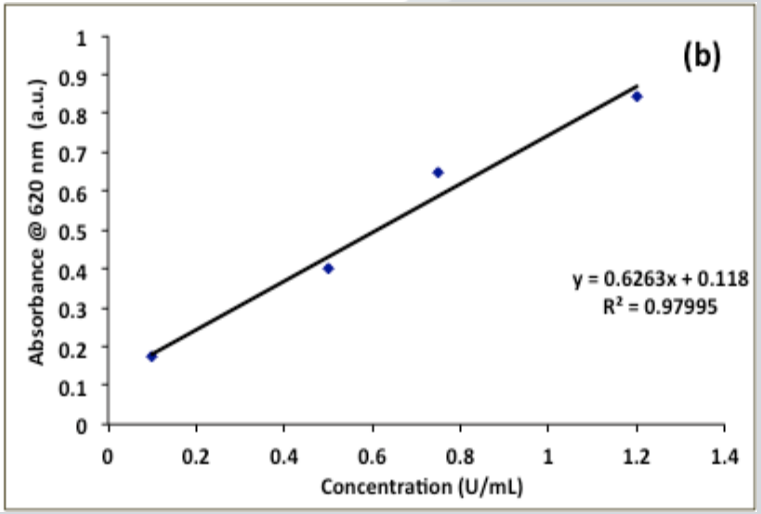
Determination of Chemo/Bio-markers using mobile platform

- Calibration curve for determination of α-amylase:
  - Calibration curve constructed using human amylase standard
  - Device optimised for determination of alpha-amylase levels within specific range (0.1 U/mL – 1.2 U/mL)
  - Clinical trials in progress.



Calibration curve of amylase using portable platform





(a) UV-Vis spectra of amylase standards. (b) Calibration curve for validation using UV-Vis

Conclusions and future applications

- *Use of Non-invasive sample for **increased frequency of patient monitoring** .*
- *Reliable Portable optical detection of both chemical and biological markers for eventual **Reduction in requirement for hospital visits** with patients able to determine levels at home.*
- ***Customisable** nature of device allows for the potential of tailoring the system (LEDs and detectors) to alternative targets that can be detected optically allowing for use with other disorders.*

Acknowledgements

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